

CLAIMS:

1. A method of producing a refolded, inactive HCV NS2/3 protease, comprising the steps of:
 - a) isolating said protease in the presence of a chaotropic agent;
 - b) refolding said isolated protease by contacting it with a reducing agent, and lauryldiethylamine oxide (LDAO) in the presence of reduced concentration of said chaotropic agent or a polar additive.
2. The method according to claim 1, wherein said LDAO is at a final concentration at, or above critical micelle concentration.
3. The method according to claim 2, wherein said LDAO is at a final concentration between 0.003% and 1%.
4. The method according to claim 3, wherein said LDAO is at a final concentration between 0.03% and 1%.
5. The method according to claim 4, wherein said LDAO is at a final concentration of 1%.
6. The method according to claim 1, wherein in step a) said chaotropic agent is selected from the group consisting of: guanidine-HCl, guanidine or urea.
7. The method according to claim 6, wherein said chaotropic agent is at high concentration between 5M and 8M.
8. The method according to claim 7, wherein said chaotropic agent is guanidine or guanidine-HCl, each at a final concentration of 6M or urea at a final concentration of 8M.
9. The method according to claim 8, wherein said chaotropic agent is 6M guanidine-HCl.
10. The method according to claim 1, wherein in step b), the chaotropic agent or polar additive is selected from the group consisting of: guanidine, guanidine-HCl, urea and arginine-HCl.
11. The method according to claim 10, wherein guanidine-HCl or arginine-HCl is used.
12. The method according to claim 11, wherein arginine-HCl is used.
13. The method according to claim 12, wherein said arginine-HCl is at a final concentration between 0.25M and 2M.
14. The method according to claim 13, wherein said arginine-HCl is at a final

concentration between 0.5M and 1M.

15. The method according to claim 14, wherein said arginine-HCl is at a final concentration of 0.5M.
16. The method according to claim 1, wherein the reducing agent is selected from the group consisting of TCEP and DTT.
17. The method according to claim 16, wherein the reducing agent is TCEP at a final concentration of 5mM.
18. The method according to claim 1, wherein said protease is isolated from cellular inclusion bodies.
19. The method according to claim 1, wherein said refolding is carried out by dialysis or by gel filtration to yield a purified NS2/3 protease.
20. The method according to claim 19, wherein said refolding is carried out by gel filtration.
21. The method according to claim 1, wherein said NS2/3 protease is the full length NS2/3 protease or a truncation thereof having as its N-terminal residue any one amino acid from amino acid 810 to amino acid 906.
22. The method according to claim 21, wherein said NS2/3 protease has the minimal amino acid sequence from residues 904 to 1206 of the HCV 1b-40 full-length NS2/3 protease.
23. The method according to claim 22, wherein said NS2/3 protease is consisting of a truncated NS2/3 protease as defined according to SEQ ID. NO: 10.
24. A method for producing an active NS2/3 protease further comprising:
 - c) diluting said refolded inactive NS2/3 protease as defined in claim 1, in a medium containing an activation detergent to induce auto-cleavage of said NS2/3 protease.
25. The method according to claim 24, wherein said LDAO is diluted at a final concentration equal or below 0.1%
26. The method according to claim 24, wherein in step c) glycerol is further added.
27. The method according to claim 26, wherein said glycerol is at a final concentration of between 10% and 50%.
28. The method according to claim 24, wherein the activation detergent is selected from the group consisting of: CHAPS, Triton X-100, NP-40 and n-dodecyl- β -D-maltoside.
29. The method according to claim 28, wherein the activation detergent is at a final concentration between 0.1% and 1%.

30. The method according to claim 29, wherein the activation detergent is CHAPS.
31. The method according to claim 29 wherein the activation detergent is n-dodecyl- β -D-maltoside.
32. A method for measuring the auto-cleavage activity of a NS2/3 protease further comprising:
 - d) incubating the active NS2/3 protease produced by the method of claim 24 for sufficient time to induce auto-cleavage of the NS2/3 protease and produce cleavage products or fragments thereof; and
 - e) measuring the presence or absence of uncleaved NS2/3 protease, cleavage products or fragments thereof.
33. The method according to claim 32, wherein step d) is carried out at a temperature between 15°C and 30°C.
34. The method according to claim 33, wherein step d) is carried out at a temperature between 15°C and 25°C.
35. The method according to claim 34, wherein step d) is carried out at room temperature.
36. An assay for screening a potential inhibitor of the auto-cleavage activity of an active NS2/3 protease comprising:
 - a) carrying out the method according to claim 32 in the presence of, or absence of the potential inhibitor;
 - b) comparing the amount of uncleaved NS2/3 protease, cleavage products or fragments thereof, in the presence of, or absence of the potential inhibitor.
37. An isolated polypeptide consisting of a full length NS2/3 protease or a truncation thereof having as its N-terminal residue any one amino acid from amino acid 810 to amino acid 906.
38. An isolated polypeptide consisting of a truncation of an HCV NS2/3 protease, wherein said truncated NS2/3 protease has the minimal amino acid sequence from residues 904 to 1206 of the HCV 1b-40 full-length NS2/3 protease.
39. An isolated polypeptide consisting of a truncated NS2/3 protease as defined according to SEQ ID. NO: 4 or 10.
40. An isolated polypeptide consisting of an amino acid sequence that has 90% identity over its length compared to the polypeptide according to claim 39.
41. An isolated polypeptide consisting of a truncated NS2/3 protease selected from the

group consisting of: a sequence as defined according to SEQ ID. NOs: 11, 12, 13, 14 and 15.

42. A composition comprising an isolated HCV NS2/3 protease selected from full length NS2/3 protease, a truncation thereof or a sequence as defined according to SEQ ID NOs: 2, 4, 10, 11, 12, 13, 14 and 15, wherein said protease is in a solution comprising a sufficient concentration of LDAO to prevent auto-cleavage of said protease.
43. A peptide having the sequence SFEGQGWRLL (SEQ ID NO:21).
44. A peptide according to claim 43, wherein said peptide is a NS2/3 protease inhibitor.